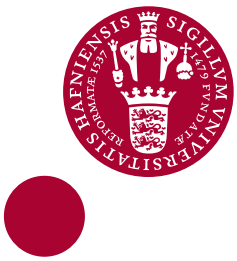

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Effect of fox, pig, sheep, and poultry bile on the establishment of domestic and sylvatic species of *Trichinella* in rats

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SUMMARY

Most sylvatic species of *Trichinella* are known to have poor infectivity to rats, but in the present study oral administration of bile from other hosts appeared to modify this infectivity. A total of 75 rats were inoculated in groups of 25 with 3 species of *Trichinella* (*T. spiralis*, *T. nativa*, and *T. nelsoni*) and each group of rats was given *per os* daily doses of bile from pig, sheep, chicken and fox respectively (4 × 5 rats). As a control 1 group of 5 rats was given daily doses of water. Whereas, the addition of bile did not increase the establishment of *T. spiralis*, fox bile had a significant positive effect on the establishment of muscle larvae of *T. nativa* and *T. nelsoni*. Addition of bile to cultures of the same *Trichinella* species had an overall negative effect on the *in vitro* survival of larvae. The present observation that carnivore bile favours the establishment of sylvatic *Trichinella* may explain why carnivores are equally receptive to all *Trichinella* species.

Key words: *Trichinella spiralis*, *Trichinella nativa*, *Trichinella nelsoni*, bile, rats.

INTRODUCTION

Foxes have a high susceptibility to all species of *Trichinella* spp. (Kapel, 2000) and as a main reservoir in Europe (Pozio, 1998) they are often used in the role of indicator population for monitoring wildlife trichinellosis. This uniform high infectivity of the *Trichinella* species is markedly different from what is found in rodents and herbivorous animals where domestic *Trichinella spiralis* has a significantly higher infectivity than sylvatic species (Kapel, 2000). The mechanisms underlying these differences have not been investigated in detail up to now. It is known that chickens are resistant to infective *T. spiralis* larvae and this resistance has been linked to the deleterious effects that extracts from the small intestine, pancreas, and bile of chickens have on the larvae (Barriga, 1981). Other authors (Smyth, Gemmell & Smyth, 1969) have found that differences in the composition of bile between definitive host species may influence host specificity in *Echinococcus granulosus*. The objective of the present study was to evaluate the effect of bile from fox, pig, sheep, and poultry on the infectivity to rats of domestic and sylvatic *Trichinella* species. In addition, the *in vitro* survival of muscle larvae was monitored in cultures where bile from the same animals was added.

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MATERIALS AND METHODS

Species and source of muscle larvae and bile

The *Trichinella* species used were *T. spiralis* (ISS004), *T. nativa* (ISS042), and *T. nelsoni* (ISS037), which have been maintained by serial passages in mice. Larvae were released from thoroughly minced mouse muscle tissue by digestion (1 L H_2O, 10 g 1 : 10 000 IU Pepsin, 10 ml of 37% HCl) for 1 h at 37 °C.

Bile was collected from foxes, pigs, sheep, and chicken and stored at −80 °C prior to experimentation.

In vivo *study*

A total of 75 female inbred 10-week-old Wistar rats were inoculated *per os* through a feeding needle with 1000 larvae in 3 groups of 25 rats with respectively *T. spiralis*, *T. nativa* and *T. nelsoni* and divided further in 5 groups of 5 rats. Four groups received diluted bile from fox, pig, sheep or chicken respectively and 1 control group received water. For 21 days, rats were administered daily portions (0·5 ml) of bile (diluted 1 : 2·6 in tap water) or water respectively. All inoculations during the experiments were done after anaesthesia with CO_2. The rats were sacrificed

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Table 1. Reproductive capacity index (RCI: larvae recovered/larvae inoculated) in rats

	T. spiralis		*T. nativa*		*T. nelsoni*	
Inoculum	RCI	Mean ± S.D.	RCI	Mean ± S.D.	RCI	Mean ± S.D.
Fox bile	13·770	66·963 ± 93·84	0·027	0·045 ± 0·03	0·014	0·0186 ± 0·0097*
	15·300		0·016		0·013	
	33·525		0·093		0·010	
	38·520		0·065		0·034	
	233·700		0·024		0·022	
Pig bile	18·150	9·950 ± 8·41	0·000	0·000 ± 0·00	0·004	0·0038 ± 0·0013
	10·350		0·000		0·005	
	1·350				0·003	
					0·002	
					0·005	
Sheep bile	5·400	15·510 ± 8·35	0·000	0·000 ± 0·00	0·005	0·0024 ± 0·0018
	17·400		0·000		0·003	
	8·250		0·000		0·002	
	22·650		0·000		0·000	
	23·850		0·000		0·002	
Chicken bile	12·825	13·894 ± 4·24	0·000	0·000 ± 0·00	0·000	0·000 ± 0·00
	18·000		0·000		0·000	
	7·538		0·000		0·000	
	17·213		0·000		0·000	
Water (Control)	12·150	12·435 ± 10·00	0·000	0·000 ± 0·00	0·000	0·000 ± 0·00
	3·375		0·000		0·000	
	3·300		0·000		0·000	
	27·300		0·000		0·000	
	16·050		0·000		0·000	
					0·000	

* Significant difference ($P<0·001$) with other RCI values in the same column.

5 weeks p.i. and the reproductive capacity index (RCI: larvae recovered/larvae inoculated) was calculated for each rat.

In vitro *study*

Larvae of each *Trichinella* species were cultured *in vitro* by using the method described by Kapel & Gamble (2000). The culture medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 4500 mg glucose/l, 25 mM HEPES and antibiotics (50 U penicillin/ml + 50 μg streptomycin). For each species of *Trichinella* six 30 ml culture flasks were used. Each flask contained 500 larvae in 5 ml of medium (concentration: 100 larvae/ml). Bile from each of the study animals was added at an amount of 0·5 ml in each of the 3 flasks at a final concentration of 1 : 10 while the remaining 3 flasks served as controls. The flasks were kept at 37 °C. The number of motile (live) and non-motile (dead) larvae in sample optical areas was counted daily under a dissecting microscope at 30 magnifications, and the percentage of motile (live) larvae for each flask was calculated. Counts were done until all larvae were immotile (dead).

Statistical analysis

Proportions of motile (live) larvae of each *Trichinella* species cultured *in vitro* in the presence of each bile or not (control) were analysed using ANOVA. The method is appropriate since the binominal distribution can be approximated by the normal for large samples as in this case ($n=100$). Since interaction terms were found significant the effect of each combination was tested separately. Multiple comparisons were used to identify significant differences and tests were performed using Tukey's honest significant difference (HSD) test (Miller, 1981). The infectivity (RCI) of each *Trichinella* species to rats administered with bile or water (control) was analysed using ANOVA as well. The Analysis was performed using SPSS v. 11.

RESULTS

In vivo *study*

The infectivity (RCI) of the 3 *Trichinella* species to rats administered with bile or water is presented in Table 1. Infection was established in all groups of rats inoculated with *T. spiralis*, but no significant differences in RCI were observed between the various groups of rats administered with bile or water. Infection with *T. nativa* was established only in the group of rats administered with fox bile. Infection with *T. nelsoni* was established in the groups of rats administered with fox, pig, and sheep bile whereas RCI was significantly higher ($P<0·001$) only in the group of rats administered with fox bile.

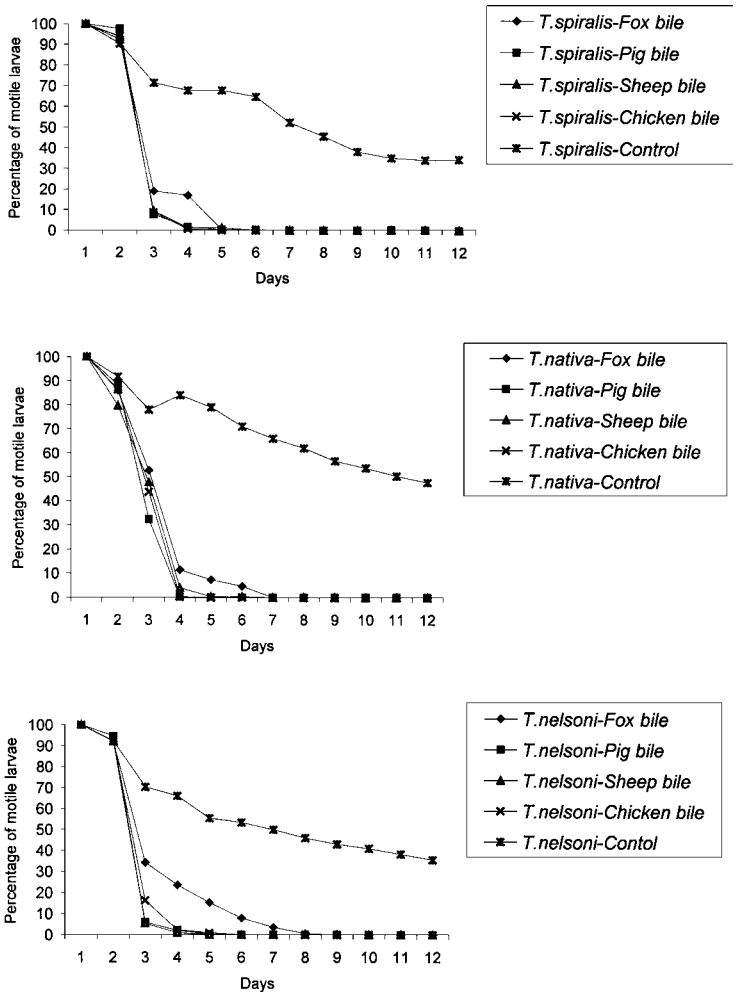

Fig. 1. Percentages of motile (live) *Trichinella spiralis*, *Trichinella nativa* and *Trichinella nelsoni* larvae cultured in the presence of bile from study animals.

In vitro *study*

The percentages of motile (live) larvae of the 3 *Trichinella* species cultured in the presence of bile from any animal were significantly lower ($P<0{\cdot}001$) compared to their respective control cultures (Fig. 1). On the other hand, the percentage of motile (live) *T. nelsoni* larvae cultured in the presence of fox bile was significantly higher ($P<0{\cdot}01$) than in the presence of pig bile ($P<0{\cdot}01$) or sheep bile.

DISCUSSION

The mechanisms that are responsible for differences in host susceptibility to various *Trichinella* species are only poorly understood, but the enteric phase of the life-cycle is a potential point at which the interaction between host and parasite can be stopped (Kapel, 2000). During the intestinal phase of infection both larvae and adults come in contact with bile, and the present study suggests that the composition of the bile influences the infectivity as well as the *in vitro* survival of larvae.

Several authors have demonstrated that *T. nelsoni* and *T. nativa* have indeed very low infectivity in rats and that *T. spiralis* is highly infective (Pozio *et al.* 1992; Kapel, 2000; Malakauskas, Kapel & Webster, 2001). Whereas the present study did not demonstrate a further increase in the infectivity of *T. spiralis* with addition of bile, the reproduction of *T. nativa* and *T. nelsoni* was obviously improved by addition of the fox bile. Although preliminary, this observation may reflect that most sylvatic species of *Trichinella* initially have been adapted to the carnivore gut environment, and that the domestic *T. spiralis* has developed an ability to reproduce without specific carnivore stimulation.

The results of the *in vitro* study show that bile, regardless of its origin, had a deleterious effect on the motility of the 3 *Trichinella* species. Although several factors could be responsible for the increased mortality, the bile may stimulate the initial moulting processes leading to incomplete cuticle transformations that increase mortality.

The mechanism explaining the action of bile on the infectivity of *Trichinella* sp. is not known. Possible actions of bile on *Trichinella* larvae are, the digestion of larvae as it has been shown in the case of chicken bile (Barriga, 1981), the inhibition of larvae by

immune secretory IgA from bile (Jacqueline *et al*. 1981), the alteration of the parasite's behavioural and nutritional status by exposure to bile (Stewart *et al*. 1987), the structural reorganization of the surface coat of larvae by exposure to bile (Smith, Selkirk & Gounaris, 2000), and the modification of tyrosine phosphorylated proteins on the larval cuticle by incubation of the larvae with bile (Allegretti *et al*. 2001). Nevertheless, all the above mechanism explain the detrimental effect of bile on *Trichinella* larvae. The exact mechanism explaining the favorable effect of the feeding of carnivore bile on the reproductive capacity of sylvatic *Trichinella* adult worms *in situ* in the small intestine of the rats is not known. Apparently, the carnivore bile could survive the digestion by stomach enzymes and the absorption in the small intestine in order to impose its favorable effect on the parasite as observed in the present study.

In conclusion, carnivore bile appears to favour the establishment of sylvatic *Trichinella* spp.

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